SHORTER ARTICLES AND DISCUSSION

AN ATTEMPT TO MODIFY THE GERM PLASM OF ŒNOTHERA THROUGH THE GERMINATING SEED

WHEN a new character appears in a homozygous race or species it may be either a mutation or an acquired character. If a mutation it has been produced because the germ plasm has in some way been affected and the succeeding generations may be expected to show the same variation. If an acquired character it will be present for a single generation and is then lost unless the cause that produced the somatic change also modified the germ plasm in such a manner that it may develop the same character, in succeeding generations. By "acquired characters" is meant any and all changes that are wrought in the soma of the organism by the environment considered in its broadest sense. It is the creed of modern biology that acquired characters are not inherited unless the environmental influences also play on the germ cells even while focused on the body tissues, producing at the same time potential alterations in the former and visible changes in the latter.

The causes of mutation are in dispute. It is fairly obvious, however, that in the reported instances of mutation the variations arose as the result of changes in the germ plasm. A mutation, therefore, has its origin from within and this origin has no very evident connection in any way with external conditions. If a case of an acquired character is shown to be inherited it is clear that the germ plasm of the organism must have been affected. The stimulus to change, therefore, in contrast with the cause of mutation, would have come from without. While the end result—the alteration of the germinal constitution—is the same in both cases, the method or cause by which it is brought about is different. From this point of view, the relative value in evolution of mutation and of hereditary acquired characters is open to various interpretations. I think that in the case of mutations it may soon be possible to demonstrate that some of the so-called examples of "mutations" are due to or are associated with irregularities of karyokinesis. It is not at all inconceivable that outside conditions producing ac-

quired characters may at the same time effect chromosome structure and behavior in the germ plasm. The cause for unusual activity on the part of the chromosomes in either case may, however, well be totally different.

Interesting as are the effects of natural influences on the germ plasm, of greatest importance is the problem whether or not modifications may be produced and controlled artificially. The fact that experimental research is tending to show the specificity of certain chemicals for various organisms or parts of organisms makes hopeful the outlook for finding a specific or many specifics that will act upon the mechanism of heredity or different parts of that mechanism. At present our investigations can only be empirical, trying this or that method more or less blindly. Considerable work in this field has already been done. Without entering into a full discussion of the numerous investigations, it may be said that alcohol, temperature, humidity, ether, zinc sulphate and radium have been used in attempts to alter the germinal constitution. Changes indeed have been produced in some cases but the effects have been generally physiological in nature either interfering with development or influencing color, length of hair, etc. In all cases where the offspring resembles the altered parents the results have been readily interpreted on the assumption that eggs or embryos are influenced at the same time as were the forms producing them. MacDougal reports having produced changes in one of the œnotheras through ovarial injections. Although it may be possible that he produced modifications, the plant he selected for experimentation was unfortunate since the natural variability of the œnotheras is in most cases great, and the gametic purity of his material was not clearly demonstrated.

In many ways plants offer the most favorable material for the study of experimental variation. Many parts of the plant are adapted to experimentation. The pollen may be subjected to treatment, the ovaries may be injected with chemicals or otherwise handled, the seeds offer themselves to various manipulations, many stages of growing plants are available for experimentation, a variety of experiments may be conducted and, if there are numerous branches, controls may be maintained on the same plant. Coupled with these advantages are possibilities of cultivating pure lines for many generations. It would be difficult indeed to find an animal about which all of the above statements could be made.

Two methods of inducing germinal variation seem practical. The first is the treatment of the germ cells. MacDougal's ovarial injections lie in this class. The other method is to attack the buds or growing points of the stems. I do not at present believe that exact results can be expected from ovarial injections since the ovules are generally so tightly packed that fluids can not readily circulate and it is impossible to know the quantity and the strength of the fluid that may reach the germ plasm. The treatment of pollen may offer greater possibilities unless unforeseen technical difficulties are encountered. By subjecting growing points to treatment the germ plasm may more readily be affected and the complete chromatin equipment may be placed under the influence of the materials used. If germinating seeds or seedlings are immersed in a given chemical solution we may have reason to expect that the cells of the growing points are probably in contact with the solution or some derivative of it. If the chromosomes of a growing point can be influenced it is possible that the organs that are developed from this point may be altered.

Two years ago, in connection with a program of study dealing in general with problems of development, I had the opportunity of making some experiments on seeds and seedlings of *Œnothera biennis* L. (the Dutch *biennis*) from a pedigreed line which had been inbred for at least eight generations. *Œnothera biennis* L. is one of the most stable species so far studied in this genus and its rare "mutations" are known from the research of Professor DeVries. Consequently it seemed justifiable to anticipate that any results obtained through experimental treatment may readily be recognized. To Dr. Bradley M. Davis I am indebted for suggesting *Œnothera biennis* as a favorable plant for study, for many pedigreed seeds and for the complete freedom of his garden. Without his aid in the study of the plants and without his advice I should have made little progress. In preliminary studies of this sort all the in-investigator can hope to accomplish is to determine certain solutions which produce suggestive effects. The results of my studies are here brought together in hope that they may be of some help to other workers.

As has been pointed out, studies of this character must at present be largely empirical. The problem is to find chemicals that will modify the structure of the germ plasm or bring about irregularities in the distribution of the chromosomes. Some of

the chemicals used are those frequently employed in fixing fluids; others were selected for various reasons.

As shown in the List of Experiments the seeds and seedlings were soaked for varying lengths of time in the solutions. The material was either thoroughly washed before being placed on moist filter paper in petri dishes to complete germination or it was placed on paper which had been moistened with the same solution as that in which the material had been soaked. Since the seeds of *Œnothera biennis* are about 96 per cent. viable, seed sterility was not an important factor in the results of the experiments. Dr. Davis's large cultures under normal conditions were used as controls. About one hundred seeds were used in each experiment.

LIST OF EXPERIMENTS

Fluids	Percentage	Seeds or Seedlings	Time in Solution	Percentage of Germination
Acetic acid	0.125	Seeds	4 days	25
	0.125	Seedlings	3 days	
	0.625	Seeds	29 days	40
Butyric acid	0.25	Seeds	34 days	40
	0.5	Seeds	34 days	0
	1.0	Seedlings	1 day	
	1.0	Seeds	5 days	0
Chloral hydrate	0.75	Seedlings	5 days	
	0.75	Seeds	34 days	50
	0.375	Seeds	24 days	0
	0.187	Seeds	24 days	50
Chromic acid	0.03	Seedlings	11 hours	
	0.015	Seeds	4 days	65
	0.015	Seeds	3 days	60
	0.015	Seedlings	21 days	
	0.015	Seeds	3 days	60
Ethyl alcohol	5.0	Seedlings	8 days	
	5.0	Seeds	27 days	0
	1.0	Seeds	18 days	40
	0.5	Seeds	3 days	60
	0.5	Seeds	24 days	80
	0.25	Seeds	24 days	A few germinations
Methyl alcohol	1.0	Seeds	18 days	Normal germination
Amylic alcohol	1.0	Seeds	18 days	0
Butylic alcohol	1.0	Seeds	18 days	0
Propylic alcohol	1.0	Seeds	18 days	0
Zinc sulphate	10.0	Seeds	18 days	0
	5.0	Seeds	18 days	A few germinations
Strychnine		Seeds	10 days	0
Pot. bromide and iodide		Seeds	10 days	0
Ferric alum	4.0	Seeds	10 days	0
	0.5	Seeds	14 days	19

RESULTS

In the acetic-acid solutions mold grew vigorously and possibly interfered with the growing plants. The percentage of germination was low. In the young plants the cotyledons were rather more pointed than normal, although this modification was not marked. The leaves of young rosettes also appeared more narrow and pointed, but these peculiarities disappeared as the plants matured.

All the seeds and seedlings treated with butyric acid died.

Chloral hydrate produced no effect other than retarding the period of germination, reducing its percentage, and weakening the plants.

Chromic acid produced by far the most interesting results. In the various solutions used germination was prompt (about the usual three to four day period) but the percentage was materially lowered. The seedlings produced were vigorous in appearance, although the root system was in most cases stunted. There was a slight though not, I believe, significant modification of the cotyledons which were somewhat less pointed than in the type. Some of the seedlings were bright red and practically all had a reddish or pinkish tinge. Growth after planting was slow but all the plants finally developed normally.

Ethyl alcohol produced no modification of structure, although I believe that it will be worth while to continue this line of experimentation. In all cases where seeds were allowed to soak in ethyl alcohol the solution became thick and gelatinous from a substance extracted from the seeds. The percentage of germination was much reduced.

Methyl alcohol retarded germination but the resulting plants were fairly normal.

Amylic, butylic and propylic alcohols all inhibited germination in the strengths employed.

Germination was also inhibited by the solutions of zinc sulphate, strychnine, potassium bromide and iodide and by four per cent. ferric alum.

In general it may be said that the treatment of seeds and seedlings in the experiments has resulted, as in the experiments of others, in reducing the percentage of germination or in a general weakening of the plants rather than in specifically modifying the germinal constitution. The results from the experiments with chromic acid and possibly with chloral hydrate and ethyl alcohol suggest the desirability of further studies. In future

work the concentration of the agent and the length of treatment should be studied in greater detail.

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CONCERNING A MORPHOLOGICAL PREDICTION FROM DISTRIBUTIONAL DATA AND ITS SUBSEQUENT VERIFICATION[1]

ALL species of the genus *Salpa* are notable for their two alternating generations. One, known as the "solitary" generation, produces offspring by budding and the buds, when fully mature, constitute what is known as the "aggregate" generation, or, as I shall call them, *zooids*. Each zooid produces, sexually, one of the solitary generation, the embryo being nourished and carried within the body of the zooid until after it has begun to form zooids of the succeeding generation.

The relation between the two generations, however, is not simple; and as there seems to be a widespread misconception regarding this relation, it is well to correct it.

One sees the statement in much of the literature that the solitary generation is asexual, that the aggregate generation is sexual (hermaphroditic), and that the developing embryo of the solitary form is carried within the body of its mother. Such, however, is not the case; Brooks having clearly demonstrated in his classical volume "The Genus *Salpa*," that the solitary form is, in the most literal sense, a female while the zooid it produces by budding is a male. In brief, the curious sexual relationships are as follows:

The solitary form, or female, produces immature males by budding, within the body of each of which the mother tucks away one fully developed and ripened egg together with its follicle. Before fertilization the egg is suspended by means of a fertilizing duct, which opens into the cloaca, into one of the blood channels of the newly formed zooid. The spermatozoa which are drawn into the pharynx of the zooid with the sea-water, are swept past this opening by the contraction of the muscles in swimming, and some of them enter, one of which penetrates to the egg and fertilizes it. The embryo, at an early stage, pushes into the

[1] A paper read before the Western Society of Naturalists, Friday, April 6, 1917, at Stanford University.